Screening of Protein Libraries by Anchor Less Display (ALD) and FACS
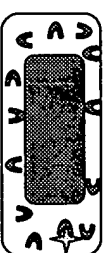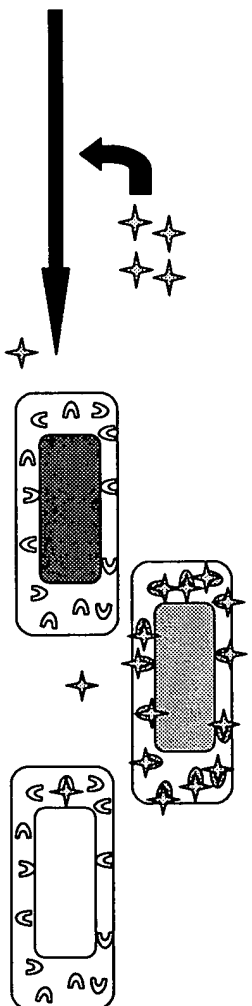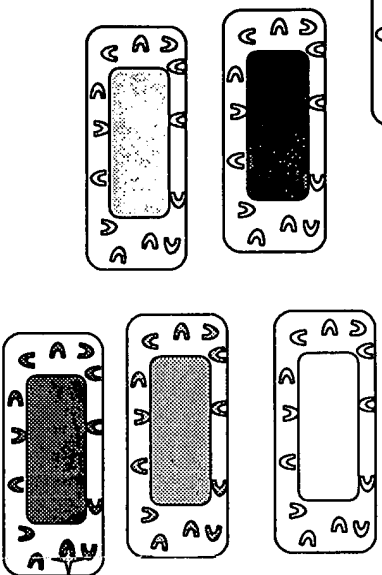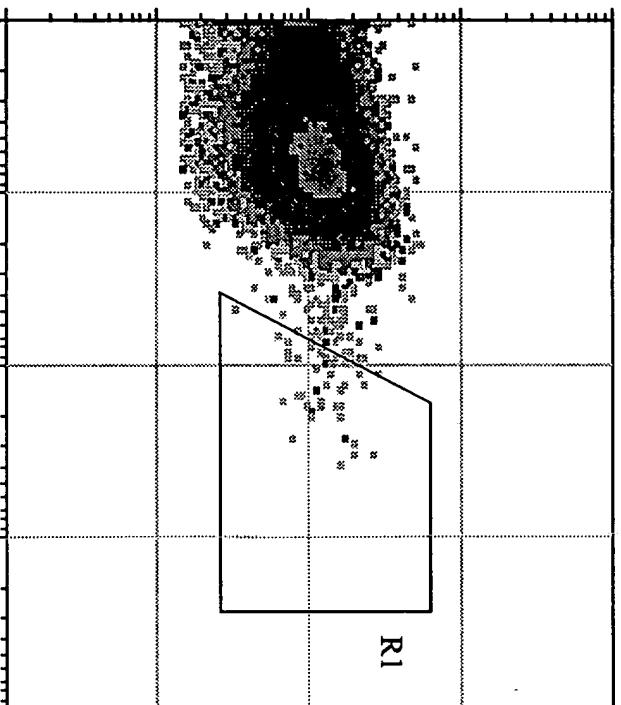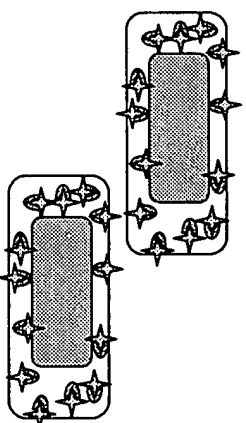
FIG. 1

Isolation of Affinity Improved Mutants by Two Rounds of Sorting

SSC Height — Light Chain Library (FL1 Height)

SSC Height — Post 1st Round Sort (FL1 Height)

SSC Height — Post 2nd Round Sort (FL1 Height)
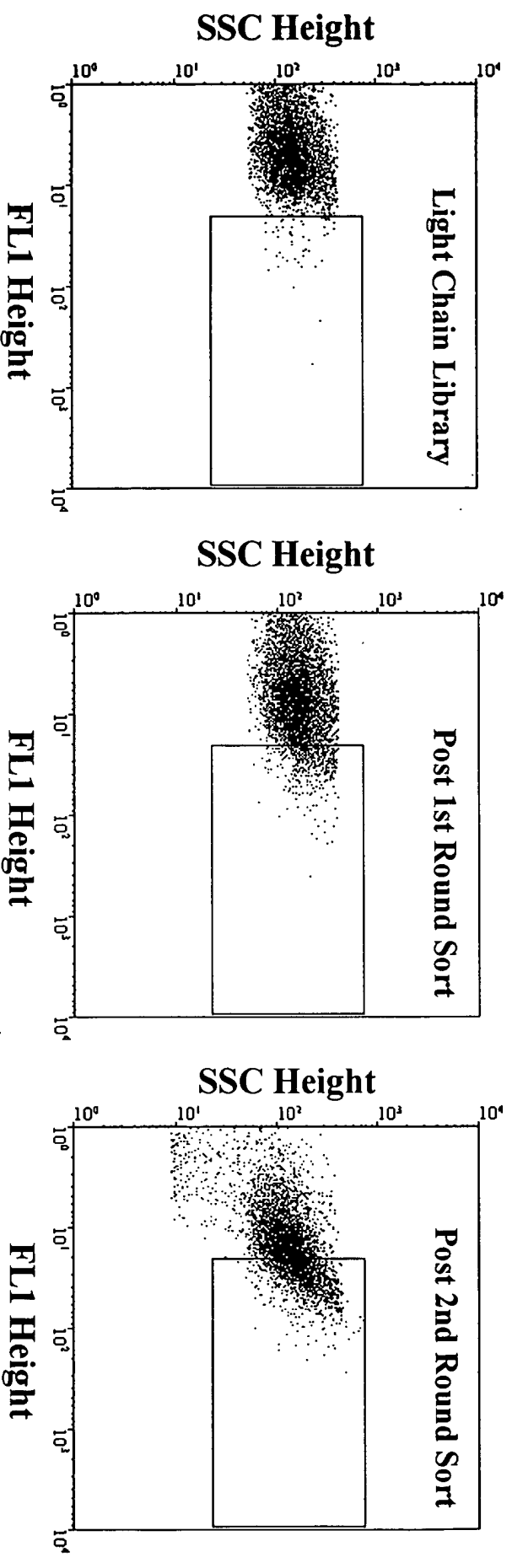
Size of the library = 2x10⁶ transformants

FIG. 2

i) 7.34

ii) 7.11

iii) 4.95

iv) 3.16

v) 27.91

vi) 15.04

vii) 122.67

viii) 54.95

FIG. 3

i) 5.74

ii) 6.01

iii) 6.17

iv) 21.24

v) 41.18

vi) 61.70

vii) 99.72

viii) 110.23

FIG. 4

i) 12.69

Events | 64 | 0
FL1-Height | 10^0 10^1 10^2 10^3 10^4

iii) 164.01

Events | 64 | 0
FL1-Height | 10^0 10^1 10^2 10^3 10^4

ii) 10.46

Events | 64 | 0
FL1-Height | 10^0 10^1 10^2 10^3 10^4

iv) 447.85
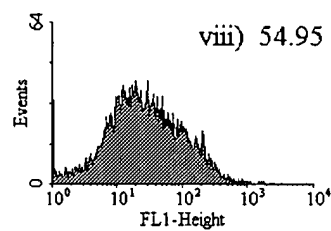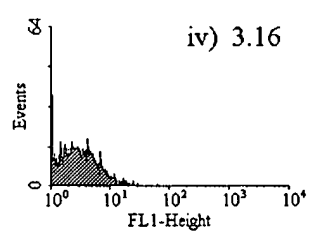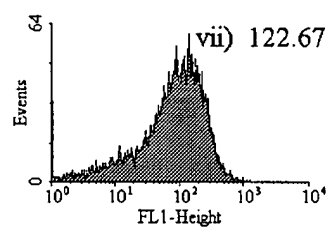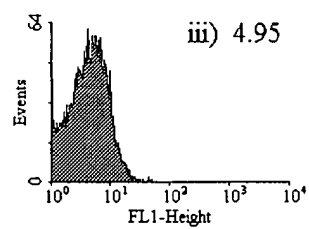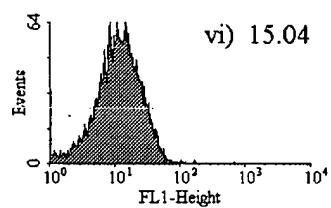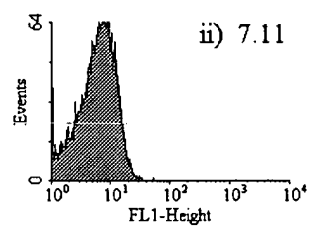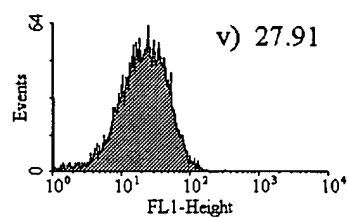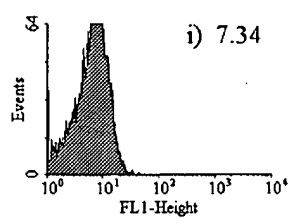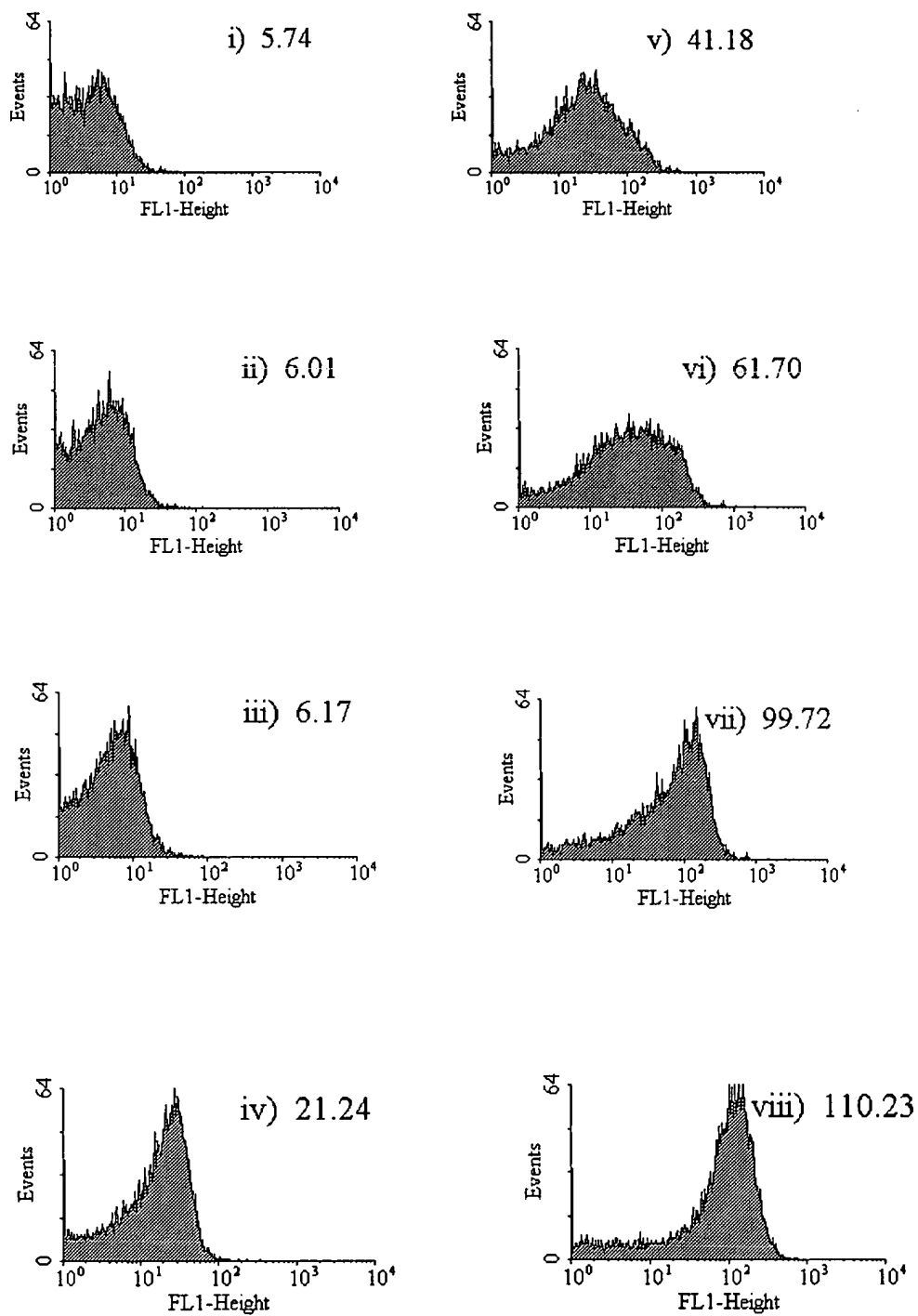
Events | 64 | 0
FL1-Height | 10^0 10^1 10^2 10^3 10^4

FIG. 5

FIG. 6A

FIG. 6B

FIG. 6C

```
                                10
GlnValGlnLeuLeuGlnSerAlaAlaGluValLysLysProGlyGluSerLeuLys
CAGGTGCAGCTGTTGCAGTCTGCAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAG
          G AG       GG G GCT  GTC       T   A  G   C     GA
          ValGlu     GlyGlyGlyLeuVal              Gly       Arg


20                              30 _____CDR1_____
IleSerCysLysGlySerGlyTyrSerPheThrSerTyrTrpIleGlyTrpValArg
ATCTCCTGTAAGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGC
C        GCA CC       T  C   C GTGA    AC  GA     A C
Leu       AlaAla      PheThr   SerAsp   TyrMetSer   Ile


    40                              _____52a_____
GlnMetProGlyLysGlyLeuGluTrpMetGlyIleIleTyrProGlyAspSerAsp
CAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGAT
    GCT  A      G    G        G TTCATAC  TAG AG A   GTAGTACC
    Ala                          ValSerTyr  SerSerSerGly   Thr


_____CDR2_____              70
ThrArgTyrSerProSerPheGlnGlyGlnValThrIleSerAlaAspLysSerIle
ACCAGATACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATC
TATAC   GCAGAC  TG GA G    GAT         CAGG     CG   AG
IleTyr   AlaAsp   ValLys    ArgPhe        Arg    AsnAlaLys


       80       82a b   c                       90
SerThrAlaTyrLeuGlnTrpSerSerLeuLysAlaSerAspThrAlaValTyrTyr
AGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACGGCCGTGTATTAC
A T ACTG  T     AAT   A        GA    GA
AsnSerLeu       MetAsn        Arg    Glu


          _____CDR3_____                    110
CysAlaArgAlaSerProSerGlyPheAspTyrTrpGlyGlnGlyThrLeuValThr
TGTGCAAGAGCTTCTCCTTCGGGGTTTGACTATTGGGGCCAAGGTACCCTGGTCACC
        ACGGG TT C
        ThrGlyPhePro
            A G AT
            ThrTyr


ValSerSer

GTCTCGAGT               FIG. 7A
```
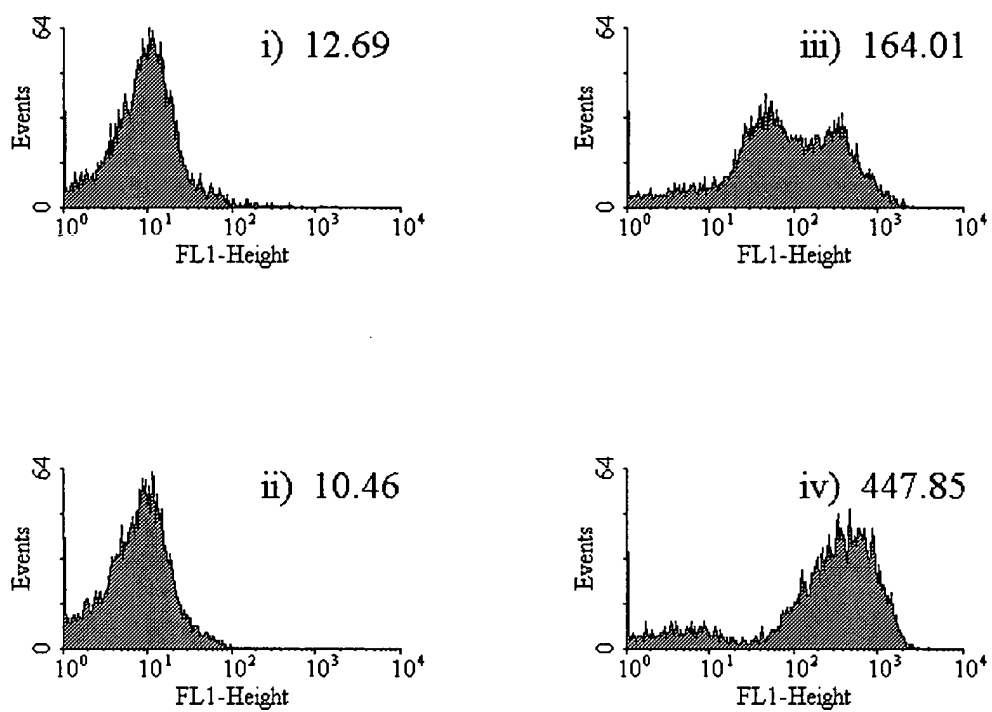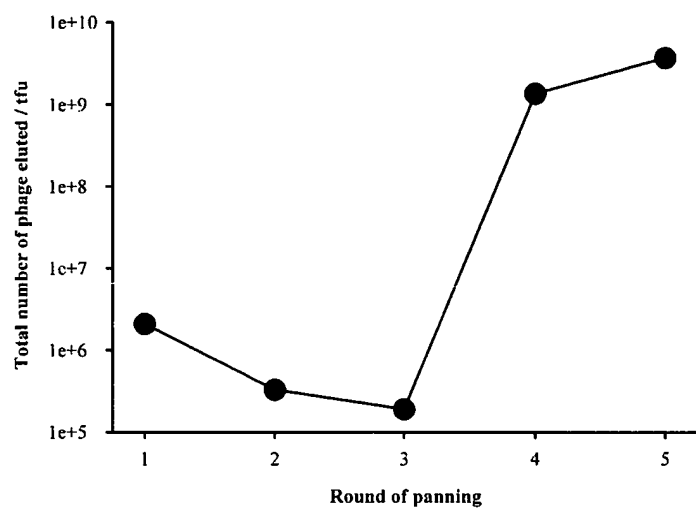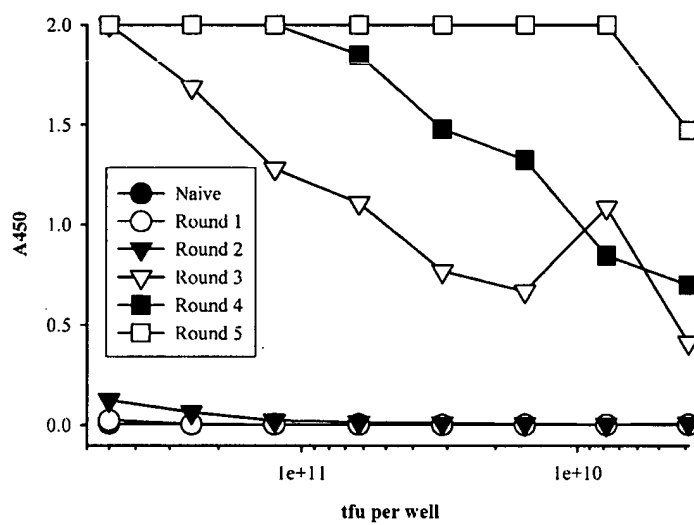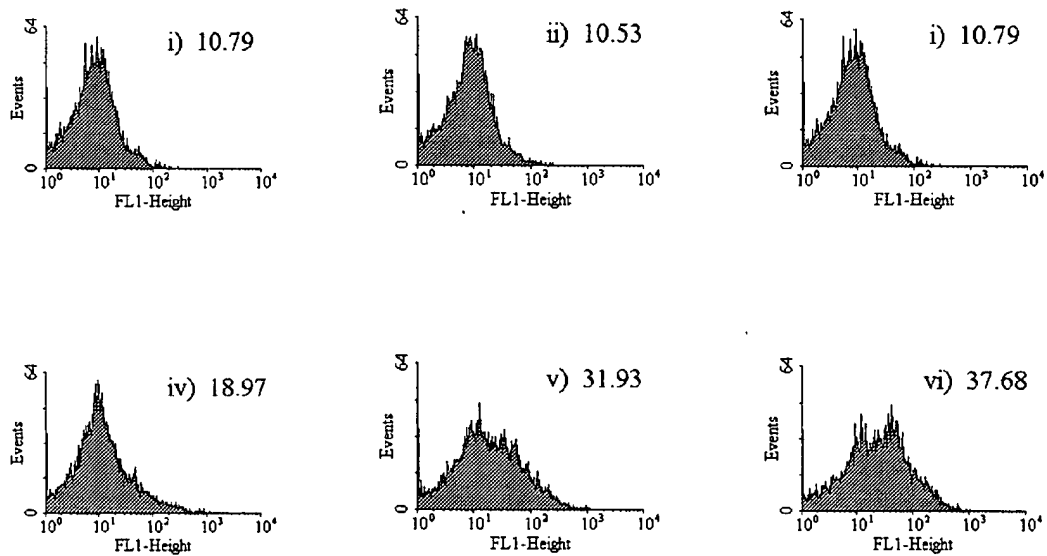
```
                                                        20
CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACC
GlnSerValLeuThrGlnProProSerAlaSerGlyThrProGlyGlnArgValThr


                          CDR1    31  a  b
ATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAATTATGTATACTGGTACCAG
IleSerCysSerGlySerSerSerAsnIleGlySerAsnTyrValTyrTrpTyrGln


      40                                        CDR2
CAGCTCCCAGGAACGGCCCCCAAACTCCTCATCTATAGGAATAATCAGCGGCCCTCA
GlnLeuProGlyThrAlaProLysLeuLeuIleTyrArgAsnAsnGlnArgProSer


          60                           70
GGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATC
GlyValProAspArgPheSerGlySerLysSerGlyThrSerAlaSerLeuAlaIle


          80                              CDR3
AGTGGGCTCCGGTCCGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGC
SerGlyLeuArgSerGluAspGluAlaAspTyrTyrCysAlaAlaTrpAspAspSer


95  a  b              100
CTGCGGGCTGTTGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA
LeuArgAlaValValPheGlyGlyGlyThrLysLeuThrValLeu
    G   G CC
    GlyGlyPro
    CTCG ---
    ProArg---
```
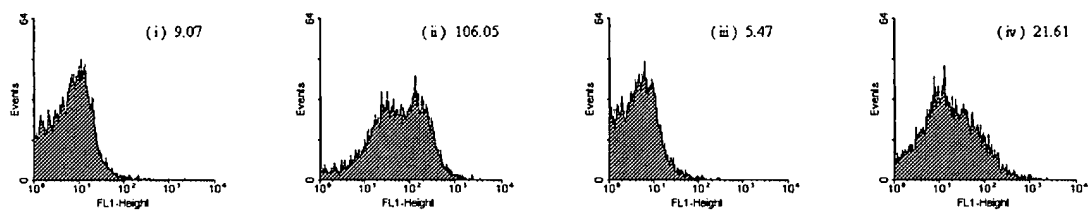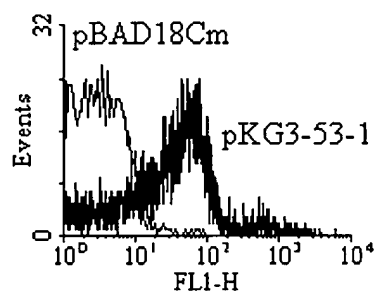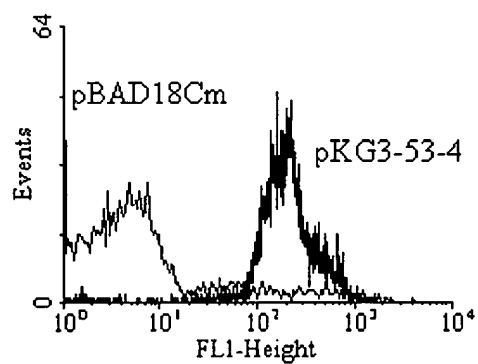
FIG. 7 B

(i) 9.07   (ii) 106.05   (iii) 5.47   (iv) 21.61

FIG. 8

FIG. 9A

FIG. 9B